REMARKS/ARGUMENTS

In response to the Office Action of January 12, 2005, Applicants request re-examination and reconsideration of this application for patent pursuant to 35 U.S.C. 132.

Claim Status/Support for Amendments

Claims 1, 39 and 44-46 have been amended. Claims 2-38 were cancelled in a previous response (filed on December 10, 2004). Claims 39-46 are withdrawn from consideration. It is understood that claims 39-46, drawn to the non-elected invention, will remain pending, albeit withdrawn from prosecution on the merits at this time. If the examined claim of the Group I invention is deemed to be allowable, rejoinder of the remaining claims (39-46) in accordance with the decision in *In re Ochiai* is respectfully requested; since the remaining claims (39-46) are limited to the use of the biopolymer marker of claim 1 (the examined claim of the elected Group I invention).

Claim 1 is currently under examination. Claims 1 and 39-46 remain pending in the instant application.

No new matter has been added by the amendments to the specification made herein.

The title of the invention has been amended to clearly

indicate the invention to which the pending claims are drawn.

In the "Background of the Invention" section a punctuation error was corrected at page 1, line 23.

The description of the reference at page 5 has been amended to correct a typographical error in the international application number. The corresponding international publication number has also been added.

The "Description of the Figures" section has been amended to add sequence identification numbers and to clearly indicate that Figures 2 and 3 show the mass spectrum profiles of the disclosed biopolymer markers.

Several protocols at pages 40-45 have been amended to properly identify trademark names (SEPHAROSE, TRITON, TRIS and EPPENDORF). The protocol titles at page 41 (lines 6 and 20), page 42 (line 12) and page 43 (lines 3 and 16) were underlined in the original disclosure and with the exception of the term "SEPHAROSE" at page 41, line 20 and page 42, line 12, do not indicate text amended herein.

The paragraph at page 46 was amended to correct grammatical errors and to indicate that the disclosed markers HP AL52706 and HP AB051484 have identical sequences (SEQ ID NO:4). Both sequences are shown in the specification as originally filed at page 46, lines 10-12. Page 46 was also amended to correct the molecular

weight of SEQ ID NO:2 to 1811.95 daltons; as the disclosed 1211 daltons is actually the correct molecular weight for SEQ ID NO:3 (see attached Declaration of Dr. George Jackowski).

In the "Detailed Description" section, the term "cerebrospinal fluid" has been added to define the abbreviation "CSF" at page 49, line 10 in order to provide explicit support for cerebrospinal fluid as recited in claim 41. "CSF" is a well known abbreviation for cerebrospinal fluid in the biochemical art. A typographical error within the same paragraph has also been amended (skill replaced skilled).

The abstract has been amended to remove the legal phraseology ("said").

No new matter has been added by the amendments to the claims made herein.

Claim 1 has been amended to explicitly claim the biopolymer marker (SEQ ID NO:2). The term "biopolymer marker" is used throughout the specification as originally filed, see, for example, page 1, line 8.

Claim 39 has been amended to clearly disclose the relationship between the presence of the claimed biopolymer marker (SEQ ID NO:2) and insulin resistance. Claim 39 has also been amended to explicitly indicate how the presence of the claimed biopolymer marker is determined from mass spectrum profiles. The changes to

claim 39 find basis throughout the specification as originally filed, see, for example, page 35, lines 14-18, page 46, lines 4-12 and Figure 1.

Claim 44 has been amended to correspond with the biopolymer marker of claim 1 (as amended herein). Support for various types of kits can be found in the original disclosure, see for example, page 36, lines 9-12 and page 47, line 8 to page 48, line 17. Claim 44 was also amended to correct a typographical error (an replaced and).

Claims 45 and 46 have been amended to provide proper antecedent basis for the term "kit" in claim 44 (as amended herein).

Restriction

At page 8 (first sentence of the third paragraph) of the Response filed on December 10, 2004 it is indicated that the claimed sequences (SEQ ID NOS:1-4) are related to Alzheimer's disease. Applicants herein note that this statement is an inadvertent typographical error as SEQ ID NOS:1-4 are related to insulin resistance (see page 46, lines 4-12 of the specification as originally filed).

The Examiner has determined that the requirement for restriction is still proper and therefore has made the requirement

final.

Applicants have claimed the biopolymer markers (SEQ ID NOS:1-4)in a Markush-type grouping indicating that SEQ ID NOS:1-4 are alternatively usable (MPEP 803.02). In contrast to Applicants' presentation of SEQ ID NOS:1-4 in a Markush-type grouping, the Sequence Election Requirement presents each of SEQ ID NOS:1-4 as unrelated, patentably distinct sequences, thus introducing a contradiction into the prosecution history. Such contradictions can potentially diminish the value of any patent that may issue from the instant application. For example, since Applicants are required to elect a Group (and a single sequence) for prosecution on the merits, one reading the prosecution history may incorrectly assume that Applicants admit that the biopolymer markers of SEQ ID NOS:1-4 are separate and distinct inventions.

Request for Rejoining of Claims

Considering that claims 39-46 are limited to the use of SEQ ID NO:2 a search of these claims would encompass this specific peptide. The instant application is related in claim format to several other applications, both pending and issued, of which serial number 09/846,352 is exemplary. In an effort to maintain equivalent scope in all of these applications, Applicants respectfully request that the Examiner consider rejoining claims

39-46 in the instant application, which are currently drawn to non-elected Groups, with claim 1 of the elected Group under the decision in *In re Ochiai* (MPEP 2116.01), upon the Examiner's determination that claim 1 of the elected invention is allowable and in light of the overlapping search. If the biopolymer marker peptide of SEQ ID NO:2 is found to be novel, methods and kits limited to its use should also be found novel.

Information Disclosure Statement

The Examiner has pointed out that the listing of references in the specification is not a proper Information Disclosure Statement. 37 CFR 1.98(b) requires a list of all patents, publications or other information submitted for consideration by the Office, and MPEP 609 A(1) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Thus, the Examiner indicates that unless the Examiner on PTO-892 form or Applicant on PTO-1449 form has cited the references they have not been considered.

The Examiner indicates that the Information Disclosure Statements filed on March 14, 2002; May 16, 2003 and October 15, 2003 have been considered as to the merits prior to the first action.

The references cited within the specification but not included

in the above-mentioned Information Disclosure Statements provide general information relating to background information and/or the state of the art, but were not deemed pertinent to the patentability of the claimed invention.

Oath/Declaration

A new oath or declaration has been required by the Examiner because while the original oath filed on February 13, 2002 contains the signature of Dr. John Marshall (inventor 2), the date of signature is omitted.

A new declaration, which has been properly executed and dated, is filed herewith.

Objections to the Specification

The Examiner notes the use of trademarks in the application (i.e. SEPHAROSE at page 40, line 23 and page 41, line 1 and TRITON at page 41, line 17 and page 42, line 8) which should be capitalized wherever they appear and be accompanied by the generic terminology. The Examiner further notes that although the use of trademarks is permissible in patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner, which might adversely affect their validity as trademarks.

Applicants have amended the specification at pages 40-45 to properly identify trademark names (SEPHAROSE, TRITON, TRIS and EPPENDORF).

The Examiner points out guidelines for the proper language and format of an abstract of a patent application and objects to the abstract of the instant application as it recites the legal phraseology "said".

The abstract of the instant application has been amended herein to remove the legal phraseology "said".

Applicants have now addressed all of the Examiner's objections and respectfully request that the objections to the specification be withdrawn.

Declaration under 37 CFR 1.132

A Declaration under 37 CFR 1.132 has been submitted herewith in order to clarify that the molecular weight of the claimed biopolymer marker (SEQ ID NO:2) is 1811.95 daltons. This Declaration has been signed by Dr. George Jackowski, the first inventor of the instant invention.

Rejection under 35 USC 112, second paragraph

Claim 1, as presented on December 10, 2004, stands rejected under 35 USC 112, second paragraph, as being indefinite for

allegedly failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner asserts that claim 1 is vague and indefinite because the biopolymer is "diagnostic" for insulin resistance. "Diagnostic" reads on not only the detection of the disease but also the analysis of the cause or nature of the disease. It is not clear how the biopolymer marker will analyze the cause or nature of insulin resistance. Applicants' intended meaning of "diagnostic" is not defined by the claims or the specification. The specification does not provide a standard for ascertaining the requisite degree and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. The Examiner suggests that the claim merely recite "detection of" insulin resistance in order to obviate this rejection.

Applicants respectfully disagree with the Examiner's assertions.

The term "diagnostic" refers to the identification of a property or characteristic, usually regarding health of an individual, such as, identifying a disease linked with the property or characteristic. It is clear from the multiple disclosures in the instant specification that the term "diagnostic" or "diagnose" refers to the identification of a disease; see, for example, page 5, lines 12-20; page 31, lines 19-22; page 32, lines 7-10; page 36,

lines 9-12; page 48, lines 3-5; page 48, line 18 to page 49, line 3; page 52, lines 4-7 and page 52, line 18 to page 53, line 6. According to the web site, dictionary. com, the term "diagnostic" relates to or refers to use in diagnosis; use in serving to identify a particular disease or to a symptom or a distinguishing feature; and/or use in serving as supporting evidence in a diagnosis (see attached definition as accessed from the internet; reference 1).

Neither the art nor the specification suggests that "diagnostic" refers to anything other than identification of a disease. Thus, Applicants respectfully submit that the Examiner has no basis for asserting that the term "diagnostic" reads on not only the detection of the disease but also the analysis of the cause or nature of the disease.

However, in the interest of compact, efficient prosecution, Applicants have amended the claim to remove the term "diagnostic".

Accordingly, Applicants have now clarified the metes and bounds of the claims and respectfully request that the above-discussed rejection under 35 USC 112, second paragraph be withdrawn.

Rejection under 35 USC 101

Claim 1, as presented on December 10, 2004, stands rejected under 35 USC 101 because the claimed invention allegedly is not supported by a either a specific, substantial, credible or asserted utility or a well-established utility.

Applicants respectfully disagree with the Examiner's contention and assert that the claimed invention has both a specific and a well-established utility.

The Examiner asserts that Applicants have disclosed in the specification that SEQ ID NO:2 is not measurable in patients having insulin resistance but is present in normal patients. However, this disclosure appears to require not only SEQ ID NO:2 but a combination of SEQ ID NOS:1-4 for the identification of insulin resistance.

Applicants respectfully assert that this statement made by the Examiner is incorrect.

No where does the specification indicate that a combination of markers (SEQ ID NOS:1-4) is a requirement for the identification of insulin resistance through the use of the disclosed methods. Page 46, lines 4-12 of the specification discloses that five markers were identified through use of the disclosed methods which are related to insulin resistance(the identified fifth fragment has a sequence identical to that of SEQ ID NO:4). Contrary to the

Examiner's assertion, each of the disclosed peptides has the ability to be independently related to insulin resistance.

At page 9 of the Office Action mailed on January 12, 2005, the Examiner asserts that SEQ ID NO:2 does not appear to be a marker for insulin resistance.

Applicants respectfully disagree with the Examiner's line of reasoning and assert that SEQ ID NO:2 is useful for diagnosis and treatment of insulin resistance since it was found to evidence a link to insulin resistance (an "asserted" utility). This asserted utility is supported by data derived from the working examples (the gel shown in Figure 1), which shows that the claimed peptide is differentially expressed between insulin resistance/diabetes and patients determined to be normal with regard to insulin resistance and diabetes.

The Examiner is reminded that an Applicant's assertion of utility creates a presumption of utility that will be sufficient to satisfy the utility requirement under 35 USC 101 (see MPEP 2107.02 III A). Thus, the requirements of 35 USC 101 are met solely by Applicants above assertion regarding the use of the claimed peptide (SEQ ID NO:2).

Additionally, it has been established that where an applicant has specifically asserted that an invention has a particular utility, the assertion cannot be simply dismissed by Office

personnel as being "wrong", even when there may be a reason to believe that the assertion is not entirely accurate (see MPEP 2107.02 III B).

Thus, Applicants respectfully assert that it is improper for the Examiner to state that SEQ ID NO:2 does not appear to be a marker for insulin resistance.

Furthermore, Applicants' statement of an asserted utility also constitutes a specific and substantial utility that is supported by the specification as originally filed (see page 1, lines 5-13; page 35, lines 14-18; page 46, lines 4-12; and Figure 1).

The claimed peptide (SEQ ID NO:2) does not evidence a link to a myriad of unspecified diseases but rather evidences a link to a specific disease, insulin resistance, thus the invention has a specific utility.

Additionally, if an invention is determined to have "real-world" value, one skilled in the art can use the claimed discovery in a manner that provides some immediate benefit to the public (as established in *Nelson v. Bowler and Crossley* 206 USPQ 881).

Advances in diagnosis and treatment of insulin resistance are highly desirable considering that insulin resistance is often a prelude to the development of clinical diabetes. Thus, advance in diagnosis and treatment of insulin resistance would greatly benefit a population which is susceptible to the development of diabetes.

The claimed peptide (SEQ ID NO:2) represents an advance in insulin resistance/diabetes research; a "real-world" use benefitting the public, which satisfies the precedent set in *Nelson*. Thus, the claimed peptide (SEQ ID NO:2) additionally has a substantial utility based upon a "real-world" use.

The Examiner lists several reasons which allegedly support her argument that the claimed invention has no utility.

The Examiner asserts that Figure 1 does not clearly identify SEQ ID NO:2 or its ability to determine normal patients as well as insulin resistance patients and further that no clear difference in up and down regulation of the marker can be determined.

Applicants respectfully disagree with the Examiner's contention.

At page 46, lines 4-12 of the instant specification as originally filed, SEQ ID NO:2 is identified as a fragment of inter-alpha trypsin inhibitor protein. As seen in the gel pictured in Figure 1, Bands #2 and #3 are identified as inter-alpha trypsin inhibitor protein. Thus, contrary to the Examiner's contention, Figure 1 does clearly identify SEQ ID NO:2.

The gel pictured in Figure 1 has 10 lanes; lane 1 (as read from the left) contains the low molecular weight standards; lane 2 contains a sample obtained from a Type I diabetes patient; lanes 3 and 4 contain samples obtained from insulin resistance patients;

lanes 5 and 6 contain samples obtained from Type II diabetes patients; lanes 7-9 contain samples obtained from patients determined to be normal with regard to insulin resistance/diabetes and lane 10 contains the high molecular weight standard. Band #2, pointed out in lane 9, was resolved from a sample obtained from a patient determined to be normal with regard to insulin resistance/diabetes and is labeled as inter-alpha trypsin inhibitor. Band #3, pointed out in lane 2, was resolved from a sample obtained from a Type I diabetes patient and is also labeled as inter-alpha trypsin inhibitor. However, Bands #2 and #3 are not located within the same molecular weight range, indicating that the inter-alpha trypsin inhibitor is changed in the pathological process of insulin resistance/diabetes. Band #3 is evident in all 5 disease samples (lanes 2-6); but is not evident in the normal control samples (lanes 7-9). Band #2 is evident in the 3 normal control samples (lanes 7-9); but is not evident in 4 of the disease samples (lanes 2 and 4-6) and is evident in 1 disease sample (lane 3). However, Band #2 is lighter in lane 3 as compared to lanes 7-9, indicating a possible decrease in expression of the inter-alpha trypsin inhibitor in the disease state. Thus, again contrary to the Examiner's contention, a clear difference in up and down regulation of the marker can be determined, and further Figure 1 does demonstrate an ability to determine patients exhibiting insulin

resistance from patients who do not exhibit insulin resistance (normal).

The Examiner asserts that it is not clear if Band #2 corresponds to SEQ ID NO:2.

At page 46, lines 4-12 of the instant specification as originally filed, SEQ ID NO:2 is identified as a fragment of inter-alpha trypsin inhibitor protein and Band #2, as pictured in the gel of Figure 1, is labeled as inter-alpha trypsin inhibitor. Thus, contrary to the Examiner's assertion, the instant specification clearly indicates that Band #2 corresponds to SEQ ID NO:2.

The Examiner further asserts that if Band #2 corresponds to SEQ ID NO:2, the data is ambiguous. Band #2 appears in all three normal samples and in an insulin resistant sample. The same Band #2 is not present in insulin resistance patients, Type I diabetes patients or Type II diabetes patients. Therefore, it is not clear how the marker will distinguish between any of the disease states.

The currently pending claims do not recite an ability to distinguish between disease states; but rather that the claimed biopolymer marker (SEQ ID NO:2) evidences a link to insulin resistance. When comparing Bands #2 and #3, as shown in the gel of Figure 1, it is evident that the claimed biopolymer marker, inter-alpha trypsin inhibitor, is differentially expressed between a disease state (insulin resistance/diabetes) and normal controls.

The differential expression of the inter-alpha trypsin inhibitor indicates that this protein may be linked to insulin resistance and/or diabetes, thus supporting the claims as currently pending.

In the search for specific biomarkers, proteins found to be differentially expressed between "disease" and "normal" are frequently identified as potential targets for diagnostics and/or therapeutics.

For example, Scott D. Patterson presents the state of the art in mass spectrometry/proteomics by summarizing the Asilomar Conference on Mass Spectrometry (see attached article, Physiological Genomics 2:59-65 2000; reference 2). This conference took place in 2000, thus coinciding with the time that the instant inventors were working to develop the instant invention.

In the disclosed method of the instant invention, proteins (as seen on a gel) that are identified as differentially expressed between a disease and a non-disease state are selected for excision (from the gel) and identification (see, for example, page 38, lines 7-11 of the instant specification as originally filed, and Figure 1). Such selection methods are common practice in the search for biomarkers of specific physiological states. For example, at page 61, right column of Patterson, several automation processes are discussed in the section titled "Automated identification of gel-separated proteins by mass spectrometry". This discussion begins

with the following statement:

"Following quantitative analysis of 2-DE patterns, the next step is the identification of all protein spots that display differential expression."

Thus, it is concluded that it is common practice in proteomics to select potential disease markers by their differential expression between a disease and a non-disease state.

Accordingly, when one of skill in the art observes the claimed peptide differentially expressed between insulin resistance/diabetes patients and patients determined to be normal with regard to insulin resistance/diabetes; one of skill in the art would connect the peptide with potential diagnostics and/or therapeutics for insulin resistance/diabetes and would immediately appreciate why Applicants regard the claimed peptide (SEQ ID NO:2) as useful, indicating that the utility of the claimed peptide (SEQ ID NO:2) is well-established.

Levels of plasma proteins, including the plasma protease inhibitor, inter-alpha inhibitor, can change in health and disease (see attached abstract of Salier et al. Biochemistry Journal 315:(Pt 1):1-9 1996; reference 3). Additionally, it has previously been shown that the levels of inter-alpha-trypsin inhibitor are low normal to decreased in the first few days of certain pathological conditions and increased thereafter to high normal values (see

attached abstract of Odum et al. Clin Chim Acta. 162(2):189-198 1987; reference 4).

At page 46 of the instant specification as originally filed, SEQ ID NO:2 is identified as a fragment of an inter-alpha trypsin inhibitor protein. One of skill in the art, considering that levels of plasma proteins can be altered from healthy levels in disease conditions, upon observation of the differential expression of SEQ ID NO:2 in insulin resistance/diabetes versus normal, would find it reasonable to believe that this peptide is somehow related to insulin resistance/diabetes.

Therefore, one of ordinary skill in the art would recognize the linkage between an altered level of plasma proteins, SEQ ID NO:2 and insulin resistance/diabetes and thus would also find the suggestion of SEQ ID NO:2 as a marker for insulin resistance entirely reasonable.

Accordingly, Applicants assert that the claimed invention has both a specific and a well-established utility and respectfully request that this rejection under 35 USC 101 now be withdrawn.

Rejections under 35 USC 112, first paragraph

Claim 1, as presented on December 10, 2004, stands rejected under 35 USC 112, first paragraph. Specifically the Examiner asserts that since the claimed invention is not supported by a

specific, substantial or credible asserted utility or a well established utility, one skilled in the art clearly would not know how to use the claimed invention.

It has been established by prior arguments in the instant Response that the claimed invention has both a specific and a well established utility. Therefore, Applicants respectfully request that the Examiner now withdraw the rejection under 35 USC 112, first paragraph which was based upon the rejection under 35 USC 101.

Claim 1, as presented on December 10, 2004, stands further rejected under 35 USC 112, first paragraph, as allegedly failing to comply with the enablement requirement. The Examiner asserts that the claim contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Examiner makes the following assertions:

Claim 1 is directed to a biopolymer consisting of SEQ ID NO:2 diagnostic for insulin resistance. The Examiner contends that the specification does not support this assertion. The specification (in particular page 46-47) and figure 1 do not definitively

correlate the claimed marker consisting of SEQ ID NO:2 to insulin resistance. The specification recites that the biopolymer consisting of SEQ ID NO:2 was found in the serum of patients suffering from insulin resistance (see page 46), but the specification does not contain any data supporting this contention and the figures do not identify SEQ ID NO:2 as a marker for insulin resistance. Therefore, it is unclear how SEQ ID NO:2 was identified as "notable" or how it was deemed "evidentiary" of a disease state (insulin resistance). There is nothing in the disclosure that would enable one to choose SEQ ID NO:2 as a notable sequence among an infinite number of possible proteins or peptides present in a patient sample.

Applicants respectfully disagree with all of the Examiner's assertions.

Although Applicants believe that the instant specification, as originally filed, fully supports the claim that an isolated peptide consisting of SEQ ID NO:2 is diagnostic for insulin resistance, in the interest of compact, efficient prosecution, Applicants have removed the term "diagnostic" from the claims and note that the isolated peptide consisting of SEQ ID NO:2 is linked to insulin resistance.

According to the web site, dictionary.com, the term "linked" refers to the condition of being associated with or connected to

(see attached document as accessed from the internet; reference 5). The instant specification fully supports a connection and/or an association of the claimed peptide with insulin resistance. The instant specification states at page 35, lines 14-18 that an objective of the invention is to evaluate samples containing a plurality of biopolymers for the presence of disease specific biopolymer marker sequences which evidence a link to at least one specific disease state.

The "test of enablement" is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the prior art without undue experimentation (see MPEP 2164.01).

Furthermore, the decision in *In re Brandstadter* (179 USPQ 286; MPEP 2164.05) has established that the evidence provided by applicant (to overcome an enablement rejection) need not be conclusive but merely convincing to one of skill in the art.

Applicants respectfully submit that the instant specification provides sufficient evidence to convince one of skill in the art that the claimed peptide (SEQ ID NO:2) is linked and/or associated with insulin resistance.

Claim 1 has been amended to specifically recite an isolated peptide consisting of SEQ ID NO:2, a peptide which the instant specification identifies as related to insulin resistance. Claim

1, as amended herein, does not recite that the claimed isolated peptide is diagnostic for insulin resistance, nor does it recite that the claimed isolated peptide is related to insulin resistance, even though Applicants believe that the specification, as originally filed, fully supports both of these recitations. Furthermore, the phrase "consisting of" is closed language and excludes any element, step or ingredient not specified in the claims (see MPEP 2111.03). Thus, the scope of claim 1 is limited to this specific peptide.

The gel shown in Figure 1 demonstrates that the molecular weight of the claimed peptide changes in the disease state (Band #3 compared to Band #2). Thus, a difference is seen between two comparable samples, suggesting that the differentially expressed peptide is linked to insulin resistance.

The specification, as originally filed, does provide a precise protocol on how to analyze the data obtained from the disclosed method. Page 25, line 16 to page 26, line 2 of the instant specification discloses a general outline of how to carry out the disclosed methods. Page 26, lines 6-13 of the instant specification further describes how samples were compared to develop data and indicates how biopolymer marker peptides were selected as notable sequences. This passage of the instant specification also discloses how certain peptides were selected from a plurality of molecules

found within a sample and how peptides were deemed evidentiary of a disease state. Page 5, lines 12-20 also describes how biopolymer markers are evaluated according to the methods of the instant invention. Page 46, lines 20-22 of the instant specification clearly states the steps of the invention include obtaining a sample from a patient and conducting an MS analysis (mass spectrometry) on the sample. Mass spectrometry is commonly practiced and one of skill in the art would know how to analyze and obtain information from mass spectrometry profiles. It is clear that the data presented in the instant specification was obtained by carrying out mass spectrometry. Thus, Applicants assert that the specification, as originally filed, provides a precise protocol on how to analyze the data obtained by the disclosed protocol.

Additionally, Applicants respectfully submit that such protocols are common practice in the field of proteomics.

For example, Scott D. Patterson presents the state of the art in mass spectrometry/proteomics by summarizing the Asilomar Conference on Mass Spectrometry (see attached article, Physiological Genomics 2:59-65 2000; reference 2). This conference took place in 2000, thus coinciding with the time that the instant inventors were working to develop the instant invention.

In the disclosed method of the instant invention, proteins (as seen on a gel) that are identified as differentially expressed

between a disease and a non-disease state are selected for excision (from the gel) and identification (see, for example, page 38, lines 7-11 of the instant specification as originally filed, and Figure 1). Such selection methods are common practice in the search for biomarkers of specific physiological states. For example, at page 61, right column of Patterson, several automation processes are discussed in the section titled "Automated identification of gel-separated proteins by mass spectrometry". This discussion begins with the following statement:

"Following quantitative analysis of 2-DE patterns, the next step is the identification of all protein spots that display differential expression."

Thus, it is concluded that it is common practice to select potential disease markers by their differential expression between a disease and a non-disease state.

Furthermore, Applicants respectfully submit that many of the methods disclosed in the instant specification are routinely practiced by those of ordinary skill in the art attempting to identify biomarkers of particular physiological states.

For example, at page 64, left column of Patterson is a description of the SELDI approach (as discussed at the conference by Scot Weinberger) wherein defined chemical/biochemical surfaces are utilized to allow fractionation of proteins from biological

fluids in a reproducible manner. This reproducibility allows comparisons between different samples to be made. Weinberger described a search for markers of benign prostate hyperplasia that, like prostate cancer, displays elevated prostate specific antigen (PSA) levels. The fraction exhibiting a difference between these samples was able to be enzymatically digested, and a number of peptides were generated. These peptides were able to be fragmented using the MALDI-Qq-TOF(a procedure described by Ken Standing at the conference, page 62, left column of Patterson). It was found that there appears to be a difference in the relative level of seminogelin fragments between these two states (prostate cancer and benign prostatic hyperplasia), thus providing a potential differential marker.

Applicants respectfully draw the Examiner's attention to the fact that the method described by Weinberger is analogous to the method described in the instant specification. Furthermore, when interpreting data Weinberger uses the same approach to interpretation as the instant inventors in order to identify seminogelin fragments as a potential marker to distinguish between benign prostate hyperplasia and prostate cancer based on differential expression of the fragments. Additionally, Applicants respectfully point out to the Examiner that Weinberger linked differential expression of seminogelin to benign prostate

hyperplasia and prostate cancer without analysis of a sample from a control patient free of disease or analysis of a sample from a patient having another disease, which is not benign prostate hyperplasia or prostate cancer. Such linking of markers with disease through differential expression is commonly practiced in proteomics.

Furthermore, Applicants assert that those of skill in the art are both highly knowledgeable and skilled and it is obvious that no undue experimentation would be required for a skilled artisan to follow any of the electrophoretic, chromatographic and mass spectrometric protocols presented in the instant specification in order to use the claimed invention. One of skill in the art would be able to view a gel, such as that shown in Figure 1 from which the claimed peptide was identified (SEQ ID NO:2), and recognize a difference between two comparable samples (disease state vs. non-disease state) and further recognize that the peptides present within the gel are differentially expressed between the two sample types.

Figure 1 is a photograph of a gel showing the results of HiQ1-(Elution) column chromatography as carried out with a set of eight samples; a serum sample obtained from a Type I diabetes patient (lane 2, as read from the left); 2 serum samples obtained from patients having insulin resistance (lanes 3 and 4); 2 serum samples

obtained from Type II diabetes patients (lanes 5 and 6) and 3 serum samples obtained from patients determined to be normal with regard to insulin resistance and diabetes. Lane 1 was reserved for low molecular weight standards and lane 10 was reserved for high molecular weight standards. Band #2, pointed out in lane 9, was resolved from a sample obtained from a patient determined to be normal with regard to insulin resistance/diabetes and is labeled as inter-alpha trypsin inhibitor. Band #3, pointed out in lane 2, was resolved from a sample obtained from a Type I diabetes patient and is also labeled as inter-alpha trypsin inhibitor. However, Bands #2 and #3 are not located within the same molecular weight range, indicating that the inter-alpha trypsin inhibitor is changed in the pathological process of insulin resistance/diabetes. Band #3 is evident in all 5 disease samples (lanes 2-6); but is not evident in the normal control samples (lanes 7-9). Band #2 is evident in the 3 normal control samples (lanes 7-9); but is not evident in 4 of the disease samples (lanes 2 and 4-6) and is evident in 1 disease sample (lane 3). However, Band #2 is lighter in lane 3 as compared to lanes 7-9, indicating a possible decrease in expression of the inter-alpha trypsin inhibitor in the disease state.

The data presented in Figure 1, derived from the working examples, discloses that the claimed peptide (SEQ ID NO:2) is

differentially expressed between insulin resistance/diabetes and a physiological state determined to be normal with regard to insulin resistance and diabetes, thus it can be reasonably predicted that such peptide is linked to insulin resistance/diabetes. Furthermore, Figure 1 identifies SEQ ID NO:2 and the specification discloses how such a sequence was identified as a notable sequence in relation to insulin resistance.

Thus, Applicants contend that a skilled practitioner would find that the data presented in the instant specification is convincing with regard to a link between the claimed biopolymer marker peptide (SEQ ID NO:2) and insulin resistance.

Considering the above comments, it is clear that both the specification and the prior art disclose how to make and use the instant invention. Accordingly, Applicants respectfully contend that the instant invention satisfies the "test for enablement" since one skilled in the art could make or use the invention from the disclosures in the specification coupled with information known in the prior art without undue experimentation.

The Examiner makes a series of assertions regarding the enablement of subject matter which is not claimed, including the following:

The Examiner asserts that there is no correlation between the procedure for screening samples from patients suspected of having

a variety of different diseases, the presence/absence of SEQ ID NO:2; and the determination, prediction, assessment of Insulin Resistance. There is no disclosure enabling the use of the biopolymer marker with regard to regulating the presence or absence of said sequence. The disclosure is lacking any teaching for how the identified sequence will be utilized to identify therapeutic avenues and regulation of a disease state. There is no disclosure designating how the sequence could be utilized therein, enabling one of ordinary skill in the art to use the sequence in the diagnostic method.

The Examiner is reminded that all questions of enablement should be evaluated against the claimed subject matter and the focus of the examination inquiry should be a question of whether everything within the scope of the claims is enabled (see MPEP 2164.08).

Accordingly, an Applicant is not required to enable material which is not claimed. The pending claims do not recite any methods which definitively assess the incidence of insulin resistance or any other disease state. Furthermore, the pending claims do not recite any disease state other than insulin resistance, nor do the pending claims recite identification of therapeutic avenues or methods of regulating the sequence or a disease state. Thus, no teachings regarding these issues are necessary in order to provide

evidence for enablement of the pending claims.

The Examiner asserts that Applicants merely suggest that SEQ ID NO:2 is a marker for insulin resistance and that this is contrary to the teaching of Lim (US 6,660,482 B1, issued to Lim et al.) which discloses that inter-alpha trypsin inhibitor is a diagnostic marker for sepsis (see column 3, lines 18-30 and 39-53). The Examiner alleges that Applicant has not shown support for the marker in detecting insulin resistance and since the prior art teaches that the marker is used in another non-related disease (sepsis, Lim et al.); therefore, it is not clear if the marker is detecting sepsis or Insulin Resistance.

Apparently, the Examiner believes that because the inter-alpha trypsin inhibitor is involved in sepsis, it (the inter-alpha trypsin inhibitor) can not be involved in any other pathological condition.

Applicants respectfully point out to the Examiner that the fact that the prior art does not disclose the inter-alpha trypsin inhibitor as a marker for insulin resistance does not render the instant invention "unenabled" since it has been established that the mere fact that something has not previously been done clearly is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it (see MPEP 2164.02).

Applicants respectfully assert that the teachings of Lim et al. do not preclude the involvement of the inter-alpha trypsin inhibitor or any fragment thereof in insulin resistance and/or diabetes.

The Examiner asserts that the instant invention, i.e. SEQ ID NO:2 as a marker for insulin resistance, is contrary to the teachings of Lim et al.

Applicants respectfully disagree with the Examiner's assertion.

Lim et al. do not teach that the inter-alpha trypsin inhibitor or any fragment thereof can not be a marker for insulin resistance and/or diabetes. Furthermore, Lim et al. do not disclose any relationship of the inter-alpha trypsin inhibitor or fragments thereof with insulin resistance and/or diabetes. Thus, it is impossible for the disclosure of Lim et al. to be contrary to the teachings of the instant invention.

The Examiner asserts that Applicants have not shown support for the marker in detecting insulin resistance and the prior art teaches that the marker is used in another non-related disease (sepsis). Therefore, it is not clear if the marker is detecting sepsis or insulin resistance.

Applicants respectfully disagree with the Examiner's assertion.

The methods taught by Lim et al. involve detection of the bikunin chain of the inter-alpha trypsin inhibitor and the methods taught by the instant invention involve detection of a peptide fragment of the inter-alpha trypsin inhibitor. Thus, the marker of Lim et al. and the marker of the instant are not directly analogous. The gel shown in Figure 1 demonstrates the differential expression of the claimed biopolymer marker in insulin resistance/diabetes versus a physiological state determined to be normal with regard to insulin resistance/diabetes and thus, clearly supports the use of the claimed biopolymer marker in detection of insulin resistance. Neither the specification nor Figure 1 indicate that any samples were obtained from sepsis patients, thus it is also clear that the claimed biopolymer marker is detecting insulin resistance and not sepsis. Therefore, contrary to the Examiner's assertions, Applicants do show support for the claimed biopolymer marker in detecting insulin resistance.

Accordingly, Applicants respectfully submit that the Lim et al. reference is not relevant to the instant invention.

The Examiner asserts that Applicants have not set forth any supporting evidence that suggests that SEQ ID NO:2 is a unique molecular marker for insulin resistance or any other disease and the prior art teaches that disease markers are highly unpredictable and require extensive experimentation.

The guidelines for a "test of enablement" indicate that if a statement of utility in the specification contains within it a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated, 35 USC 112, is satisfied (see MPEP 2164.01(c)).

Applicants assert that SEQ ID NO:2 is linked to insulin resistance, however, do not claim that SEQ ID NO:2 is a unique marker for any particular disease or condition.

Although the prior art does not specifically recognize that the claimed SEQ ID NO:2, a fragment of the plasma protease inter-alpha trypsin inhibitor, is related to insulin resistance, it does recognize that levels of plasma proteins can change in health and disease (see attached abstract of Salier et al. Biochemistry Journal 315:(Pt 1):1-9 1996; reference 3). When one of skill in the art observes differential expression of the claimed peptide between insulin resistance/diabetes patients and patients determined to be normal with regard to insulin resistance/diabetes; one of skill in the art will connect this peptide with potential diagnostics and/or therapeutics for insulin resistance.

Thus, Applicants respectfully submit that since the specification demonstrates a link between the claimed peptide(SEQ ID NO:2) and insulin resistance and that this link connotes the use of the claimed peptide in potential diagnostics and/or therapeutics

of insulin resistance, the requirement of "how to use" under 35 USC 112, first paragraph is satisfied.

Furthermore, Applicants respectfully submit that one of ordinary skill in the art would find the suggestion of a link between the claimed peptide (SEQ ID NO:2) and insulin resistance to be reasonable.

At page 46, of the instant specification as originally filed, SEQ ID NO:2 is identified as a fragment of the inter-alpha trypsin inhibitor. Levels of plasma proteins, including the plasma protease inhibitor, inter-alpha inhibitor, can change in health and disease (see attached abstract of Salier et al. Biochemistry Journal 315:(Pt 1):1-9 1996; reference 3). Additionally, it has previously been shown that the levels of inter-alpha-trypsin inhibitor are low normal to decreased in the first few days of certain pathological conditions and increased thereafter to high normal values (see attached abstract of Odum et al. Clin Chim Acta. 162(2):189-198 1987; reference 4). One of skill in the art, considering that changes in the levels of plasma proteins such as inter-alpha trypsin inhibitor are known to occur in disease conditions, upon observation of the differential expression of SEQ ID NO:2 in insulin resistance/diabetes versus a physiological state determined to be normal with regard to insulin resistance/diabetes, would find it reasonable to believe that this peptide is related to insulin

resistance.

Therefore, one of ordinary skill in the art would recognize the linkage between SEQ ID NO:2; fluctuating levels of plasma proteins (in disease) and insulin resistance and thus would also find the suggestion of SEQ ID NO:2 as a marker for insulin resistance entirely reasonable.

The Examiner cites two articles; Tascilar *et al.* (see attached abstract, Annals of Oncology 10, Supplement 4:S107-S110 1999; reference 6) and Tockman *et al.* (see attached abstract, Cancer Research 52:2711s-2718s 1992; reference 7) which are allegedly relevant to the instant invention.

According to the Examiner, Tascilar *et al.* is an article published in an oncogenic journal reporting on diagnostic methods in the realm of disease states. The Examiner appears to have drawn a direct parallel between the diagnostic methods reported by Tascilar *et al.* and the diagnostic methods of the instant invention. The Examiner then cites two fragmented quotations from Tascilar *et al.* "...these tests should be interpreted with caution..." and "the genetic changes found in sources other than the pancreas itself (blood, stool) should be evaluated prudently". The Examiner appears to be commenting on the predictability of molecular-based assays.

Applicants respectfully disagree with the Examiner's reliance

on the article by Tascilar et al.

Applicants assert that the claimed peptide (SEQ ID NO:2) is linked to insulin resistance; a statement which is enabled by the description of methods as set forth in the specification and by data presented in Figure 1. Thus, applicants respectfully submit that the claimed method involves a simple observation of the levels of expression of SEQ ID NO:2 (as shown in Figure 1) and does not require any other evaluation of genetic changes in the organism in which the sequence is observed.

Furthermore, the study of Tascilar et al. is concerned with the evaluation of samples for genetic mutations (K-ras and p53 mutations) for early detection of pancreatic cancer (see attached abstract of Tascilar et al. Annals of Oncology 10, Supplement 4:S107-S110 1999; reference 6). It appears that Tascilar et al. suggest that protein markers may be useful for early detection of pancreatic cancer; however there does not seem to be any other reference to protein markers, thus the study of the instant inventors (drawn to protein markers and not to genetic markers) is not analogous to the study of Tascilar et al.

Accordingly, Applicants respectfully submit that the Tascilar *et al.* article is not relevant to the instant invention.

Similarly, the Examiner cites another article, Tockman *et al* (Cancer Research Supplement 52:2711s-2718s 1992; reference 7) which

is deemed to teach conditions necessary for a suspected cancer biomarker (intermediate end point marker) to have efficacy and success in a clinical application. The reference is drawn to biomarkers for early lung cancer detection, however the basic principles are applicable to other oncogenic disorders, according to the Examiner. Tockman *et al* is deemed to teach that prior to the successful application of newly described markers, research must validate the markers against acknowledged disease end points, establish quantitative criteria for marker presence/absence and confirm marker predictive value in prospective population trials. Early stage markers of carcinogenesis have clear biological plausiblity as markers of pre-clinical cancer if validated to a known cancer outcome. According to the Examiner, Tockman *et al* reiterates that the predictability of the art in regards to cancer prognosis and the estimation of life experience within a population with a disease or disorder are highly speculative and unpredictable.

Tockman *et al* is deemed to teach that the essential element of the validation of an early detection marker is the ability to test the marker on clinical material obtained from subjects monitored in advance of clinical disease and link those marker results with histological confirmation of disease.

Applicants also respectfully disagree with the Examiner's

reliance on the article by Tockman et al.

The Tockman *et al* article is concerned with early detection of lung cancer biomarkers and apparently does not discuss biomarkers for insulin resistance or diabetes.

Tockman et al. link several biopolymer markers to lung cancer in a manner analogous to that of the instant specification. Tockman et al. state at page 2712s, left column:

"A functional membrane-associated bombesin receptor recently has been isolated from human small cell lung carcinoma (NCI-H345) cells (23), and bombesin-like peptides have been found in the bronchial lavage fluid of asymptomatic cigarette smokers (24). Thus markers of growth factor expression, insofar as they reflect oncogene activation, may also hold promise for the detection of early (preneoplastic) lung cancer."

From this statement, it is clearly evident that Tockman et al. link bombesin with small cell lung cancer and associate it with potential diagnostics for small cell lung cancer. It does not appear that bombesin was "validated" and/or subjected to any "criteria" prior to this association.

Additionally, Tockman et al. state at page 2713s, left column:

"Evidence of a transformed genome, by expression of tumor-associated antigens, oncofetal growth factors, or specific chromosomal deletions has clear biological plausibility as a marker

of preclinical lung cancer."

From this statement, it appears that Tockman et al. believe that the expression of certain proteins provides evidence of a transformed genome and since this transformed genome is associated with lung cancer, it is reasonable to believe that these certain proteins are potential markers.

Such parallel reasoning between Tockman et al. and the instant specification, further supports Applicants contention that one of ordinary skill in the art would not have any difficulty seeing a link between the claimed biopolymer marker peptide (SEQ ID NO:2) and insulin resistance.

It is noted that in chemical and biotechnical applications, evidence actually submitted to the FDA to obtain approval for clinical trials may be submitted to support enablement of an invention. However, considerations made by the FDA for approving clinical trials are different from those made by the PTO in determining whether a claim is enabled (see *Scott v. Finney* 32 USPQ 2d 1115 and MPEP 2164.05)

The Examiner is reminded that the considerations made by the PTO involving clinical trials are less stringent than the considerations made by the FDA. Evidence presented by applicant to provide enablement of an invention need only be convincing to one of skill in the art and not conclusive. Thus, Applicants

respectfully submit that compliance with the "criteria" of Tockman et al. is not necessary in order to show that the instant invention is enabled.

In conclusion, Applicants claim that the differential expression of SEQ ID NO:2 between insulin resistance patients and patients determined to be normal with regard to insulin resistance evidences a link between the claimed peptide (SEQ ID NO:2) and insulin resistance; a statement which is enabled by the instant specification, as evidenced by the arguments presented herein. Applicants assert that one of ordinary skill in the art when reviewing the instant specification, given the level of knowledge and skill in the art, would recognize the link between the claimed biopolymer marker (SEQ ID NO:2) and insulin resistance and would further recognize how to use the claimed peptide (SEQ ID NO:2) as a marker for insulin resistance. Thus, Applicants respectfully request that this rejection under 35 USC 112, first paragraph now be withdrawn.

CONCLUSION

In light of the foregoing remarks, amendments to the specification and amendments to the claims, it is respectfully submitted that the Examiner will now find the claims of the application allowable. Favorable reconsideration of the application is courteously requested.

Respectfully submitted,

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di·ag·nos·tic s k)
adj.

Of, relating to, or used in a diagnosis.

Serving to identify a particular disease; characteristic.

n.

The art or practice of medical diagnosis. Often used in the plural with a singular verb.

A symptom or a distinguishing feature serving as supporting evidence in a diagnosis.

An instrument or a technique used in medical diagnosis.

* as accessed from dictionary.com

news and reports

Mass spectrometry and proteomics

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THE 15TH ASILOMAR CONFERENCE on Mass Spectrometry this October was devoted to the role of mass spectrometry (MS) in proteomics. The Asilomar Conference site is in a picturesque national park in Pacific Grove, CA, overlooking the Pacific Ocean. The conference aims to bring together scientists from a cross section of disciplines that are applying MS to an emerging field. This year, that emerging field is proteomics. The term "proteome" was coined by Wilkins et al. (17) in the mid-1990s to describe the protein complement of the genome. The term was first used to describe the 20-yr-old field of two-dimensional gel electrophoresis (2-DE) and quantitative image analysis. 2-DE remains the highest resolution protein separation method available, but the ability to identify the observed proteins was always an extremely difficult problem. MS has been integral to solving that problem. Although improvements in 2-D gel technology had been realized since its introduction, three enabling technological advances have provided the basis for the foundation of the field of proteomics. The first advance was the introduction of large-scale nucleotide sequencing of both expressed sequence tags (ESTs) and, more recently, genomic DNA. The second was the development of mass spectrometers able to ionize and mass-analyze biological molecules and, more recently, the wide-spread introduction of mass spectrometers capable of data-dependent ion selection for fragmentation (MS/MS)(i.e., without the need for user intervention). The third was the development of computer algorithms able to match uninterpreted (or partially interpreted) MS/MS spectra with translations of the nucleotide sequence databases, thereby tying the first two technological advances together. Thus MS played a key role in the passage of 2-DE/image analysis to proteomics.

As a note to readers unfamiliar with MS, the instruments are named for their type of ionization source and mass analyzer (see also Refs. 1, 11, 12). To measure the mass of molecules, the test material must be charged (hence ionized) and desolvated (dry). The two most successful mechanisms for ionization of peptides and proteins are matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI). In MALDI the analyte of interest is embedded in a matrix that is dried and then volatilized in a vacuum under ultraviolet laser irradiation. This is a relatively efficient process that ablates only a small portion of the analyte with each laser shot. Typically, the mass analyzer coupled with MALDI is a time-of-flight (TOF) mass analyzer that simply measures the elapsed time from acceleration of the charged (ionized) molecules through a field-free drift region. The other common ionization source is ESI, in which the analyte is sprayed from a fine needle at high voltage toward the inlet of the mass spectrometer (which is under vacuum) at a lower voltage. The spray is typically either from a reversed-phase HPLC (RP-HPLC) column or a nanospray device (19) that is similar to a microinjection needle. During this process, the droplets containing analyte are dried and gain charge (ionize). The ions formed during this process are directed into the mass analyzer, which could be either a triple-quadrupole, an ion trap, a Fourier-transform ion cyclotron resonance (FT-ICR), or a hybrid quadrupole TOF (Qq-TOF) type.

This Asilomar meeting provided one of the largest academic forums in the United States for the presentation and discussion of MS as it is applied to proteomics. As is obvious from the introduction, the initial role MS played was as a protein identification and characterization methodology. However, the role of MS is expanding in this field. Although a series of talks focused on the use of different kinds of MS to identify gel-separated proteins and the various automation technologies applied to perform this in high throughput, several talks also presented alternate approaches. These approaches utilized direct analysis of digested protein mixtures for either identification of the components or quantitative analysis of two different samples mixed together. Specific biological applications were also presented. As described above, a critical component of any MS approach as applied to proteomics is the computational analysis. This report will be divided to focus on these six aspects of MS in proteomics. Where references are known for some of the material presented, they are cited. The program was, however, not entirely limited to MS in proteomics. Prior to the six sections covering the conference core, the first section of this report covers those presentations that were aimed at providing an insight into broader biological and drug discovery processes.

Proteomics in biology and drug discovery. The opening lecture, given by Lee Hood (Univ. of Washington), provided an excellent overview of Genomics, Proteomics, and Systems Biology. Hood described the genome project efforts that provide four types of maps: genetic, physical, gene, and sequence. For the human genome,

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it is anticipated that 90–95% of all genes will be sequenced sometime next year. This is the first step toward what Hood described as the "Periodic Table of Life." The different approaches to genomic sequencing and microarray technologies that are able to interrogate the mRNA levels of thousands of genes at a time were described. Hood described proteomics in broad terms as the study of multiplicity of proteins. The information obtained from the various hierarchical levels of biological information (gene, protein, pathways, interconnecting pathways) must be integrated for us to be able to provide a more complete biological picture. For both microarray and proteomics, samples representing the disease process must be obtained. This means that pure cell populations must be microscopically captured from tissues and/or sorted prior to analysis. Therefore, analyses at the mRNA and protein level must be conducted at very low levels and substantial engineering opportunities exist in the biological field to provide the necessary solutions. However, generation of the data is only the first hurdle, as the analysis of the data from a systems perspective then must be undertaken. Hood presented systems biology as the challenge for the 21st century and provided a series of examples of large-scale approaches to biology, from genome sequencing of unicellular organisms, to the sequencing of the T-cell receptor locus, to cancer biology, all of which benefit from such approaches.

Three other presentations were included in the program, to provide a broader background to the utilization of proteomics in drug discovery. Doug Buckley (Exelixis) described the generic view of the drug discovery pipeline, the various "choke" points in the process, and where proteomics could play a role. Of note was the discussion of the changing patent protection landscape, during which Buckley said that full-length cDNA patents were being issued despite the existence of EST patents on portions of these genes. Buckley also predicted that functional data is expected to be required for patents beyond the inferences gained from bioinformatics. The choke points he referred to were target validation, assay development, mechanistic biology, and toxicology. Exelixis is using model organisms (*Caenorhabditis elegans*, *Drosophila*, mouse, and zebrafish) to screen for genes that disrupt/modulate pathways common between man and these organisms. Roles for proteomics included follow-up on targets (direct analysis of protein differences, proteins associated with gene products of interest), assay development [e.g., validation of hits in high-throughput screening (HTS)], and mechanistic biology (e.g, comprehensive analysis of a knockout phenotype). Most importantly, Buckley presented the bottom line that all new technologies must demonstrate their worth by concrete changes in the drug development pipeline (i.e., greater efficiency, better decisions). He predicted that proteomics could provide these benefits at the multiple restriction points referred to above.

Pharmacoproteomics, using 2-DE to profile mechanisms of drug efficacy and toxicity, was presented by Tina Gatlin (Biosource/Large Scale Biology Corporation). The synergy between mRNA expression profiling (for low-abundance gene products) and protein expression profiling (for posttranslational modifications and subcellular localization) was presented. An exception to this is the search for surrogate markers, where secreted proteins were normally the choice and in which there is no identifiable mRNA source to mirror serum or urine protein expression. The aim of their Molecular Effects Database of 2-DE patterns, obtained from livers of drug-treated rats, is to establish links between expression patterns and toxic endpoints to reveal markers for efficacy and prediction of side effects which can be used for lead selection. In disease models, the hypothesis is that the altered expression pattern could be reversed by treatment with a drug.

The closing presentation of the meeting, given by Jeff Seilhamer (Incyte), presented analyses of the precursor to proteins, mRNA. The staff at Incyte have generated very large EST libraries and from these have estimated the number of genes in the human genome to be 129,769 (based on CpG island estimates, 142,634). They are now sequencing the human genome at a rate of about 1 million reads a month on the Megabace platform with 9 sequencing runs/day. Assembly of the data is being accomplished using Linux on 1,500 CPUs (160 computers) with 75 terabytes of storage capacity. Single-nucleotide polymorphisms (SNPs) are being calculated from their sizable EST collection, and mRNA expression profiling is being achieved using their GEM microarray platform. These data are being integrated with 2-DE proteomics data being generated by their partner Oxford GlycoSciences. This integration of the technologies of genomics and proteomics forms the basis of their drug discovery approach for profiling differences between normal and diseased tissue.

Computational aspects of proteomics. Determining the masses of peptides (MS spectra) derived from enzymatic digestion of gel-separated proteins is often the first step in a mass spectrometric-based protein identification strategy. Peptide-mass mapping is the most commonly employed mass spectrometric approach for protein identification from organisms whose genome is completely sequenced (or at least for which the more abundantly expressed genes have been sequenced). The basis of the method is the matching of experimentally determined peptide masses with peptide masses calculated for each entry in a sequence database (using the specificity of the enzyme used to generate the experimental data). How well the experimentally determined masses match with the calculated masses forms the basis of the approach. Ron Beavis (Proteometrics) described how to obtain high-quality data, which even if less, are better than more low-quality data. The use of specific matrices as well as the use of standards with respect to obtaining appropriate data sets for peptide-mass mapping was addressed. Later in the day David Fenyo (Proteometrics) described how to utilize this data in a three-step process as is performed in their WWW-available program, Profound, which uses a Bayesian algorithm (**http://www.proteometrics.com**). The process is as follows:

1) assignment of monoisotopic masses to the raw data, *2*) peptide-mass search, and *3*) significance testing of the result (4). The last step was presented as the most critical because it is from this that the confidence of the match is derived. This is achieved through calculation of a score frequency function for false positives. This was derived from statistical analysis of the database being searched using random selections of peptide masses from different proteins that are then grouped as synthetic proteins and used in a peptide-mass search of the database in question. This is repeated for a variety of random selections to come up with robust statistics for false positives.

The next level of protein identification is the generation of fragment ion spectra from peptides isolated in the gas phase of the mass spectrometer (MS/MS spectra). Matching of fragment ion spectra follows the same principle as for peptide-mass mapping. Experimentally calculated masses of fragment ions (together with the intact mass of the peptide, and often the specificity of the enzyme used to generate the peptide) are matched with those calculated for isobaric peptides (i.e., same mass as experimentally determined) from entries in sequence databases. Arthur Moseley (Glaxo Wellcome) described how nanoscale capillary LC-MS/MS (where peptides are separated chromatographically before MS/MS) had been automated for identification of gel-separated proteins. The throughput of this 75 μm ID capillary system connected to a Qq-TOF mass spectrometer was 20 samples per day at levels to 30 fmol (loaded on gel) for BSA. Moseley continues to develop ultra-HPLC (in some cases combined with variable flow systems) that improve both the speed and resolution of separation. In a Glaxo Organelle Proteomics program, various approaches to protein identification were examined. A comparison of the total number of proteins identified following in situ enzymatic digestion of proteins separated by either high-resolution 2-DE or one-dimensional (usually SDS-PAGE) gel electrophoresis (1-DE) was presented. Only one or a limited number of proteins are present in each of the 2-DE spots, whereas many proteins were present in the 1-DE bands of the enriched Golgi complex. In fact, more proteins were identified from the 1-DE bands than from the 2-DE spots (see below, *Analysis of complex protein mixtures without gel electrophoresis*).

MS/MS spectra derived from tryptic digestions conducted in the presence of equal quantities of $H_2{}^{16}O$ and $H_2{}^{18}O$, when combined with subtractive analysis of the two types of spectra, allows de novo sequencing as described by Matthias Wilm (EMBL) (18). By utilizing a Qq-TOF mass spectrometer, peptides containing both COOH-terminally incorporated stable isotopes and just the isoform containing the ^{18}O could be selected for fragmentation from the mixture. Subtraction of the ^{18}O spectrum from the ^{16}O:^{18}O spectrum reveals only ^{16}O *y*-series ions. Often, a complete ion series is obtained. The method has proved feasible in their hands when 1 pmol of protein is present in the gel (1/4 of this amount can be successfully analyzed with standard digest conditions).

Automated identification of gel-separated proteins by mass spectrometry. Following quantitative analysis of 2-DE patterns, the next step is the identification of all protein spots that display differential expression. Andrew Gooley (Proteome Systems) described the approaches they are employing for quantitative analysis using 2-DE. This included the following: sample preparation (sequential detergent extraction with aminosulfobetaine-14), narrow-range immobilized pH gradient (IPG) with mini-gels for the 2nd dimension, through to the robotic system that they have codeveloped for spot excision, liquid handling (peptide extraction and reverse-phase bead cleanup and storage) and peptide-mass fingerprinting by MALDI-MS. Apart from the throughput of the robotic system, diminished contamination from keratin and more reproducible spotting of samples for MALDI-MS is a highly desirable feature of automation. Hans-Werner Lahm (Hoffmann-La Roche) described the high-throughput system they use for automated spot excision from 2-DE, digestion (with low-salt buffer to eliminate the need for cleanup), and spotting for automated MALDI-MS. Lahm also described the computational aspects of operating such a system in high-throughput mode for long periods of time, including automated database search routines for users distributed throughout the world at other Roche sites. They are investigating the use of stable isotope labeling ($^{14}N/^{15}N$) followed by mixing of each sample prior to 2-DE for direct quantitation of relative expression differences from the MALDI-MS spectra of individual protein spots. The system averages 1,000 spots to spectra per day (including downtime).

David Arnott (Genentech) described automation of in-gel digestions following analysis of differentially regulated proteins from 2-DE. Arnott described the trapping cartridge approach that was required to analyze extracted peptides from the DigestPro robot (currently 30 sample spots, but upgradeable to 96) by microcapillary LC-MS/MS. They aimed to automate as much of the sample processing as possible with automated liquid handling from the digestion robot to the data-dependent LC-MS/MS (capable of handling 40 samples per day) using an ion-trap mass spectrometer followed by auto-database searching using Sequest (3). The system is capable of analysis of subpicomolar quantities of protein from silver-stained gels.

Advances in separations and mass spectrometers. Accurate mass analysis of intact proteins using an 11.5-T FT-ICR coupled with a capillary electrophoresis (CE) instrument was demonstrated by Richard Smith (Pacific Northwest National Laboratory) as a means of proteome analysis. Through the use of stable isotope labeling of one sample and running that sample with an unlabeled sample provides the possibility to measure protein expression ratios. To identify the proteins that display different ratios, dissociation in the FT-ICR-MS to yield mass tags is possible. Having intact mass information as well as identification allows post-translational modifications to begin to be investigated. The mass accuracy obtainable by this FT-ICR-MS was said to be <0.75 ppm which allows the generation of

accurate mass tags for tryptic peptides. In many cases this may be sufficient for protein identification (at least for an organism like *C. elegans*). In some cases, MS/MS may be required, but once performed it would not have to be repeated. Another possibility is the identification of cysteine-containing peptides at a mass accuracy of 1 ppm, which was said to be sufficient for identification. Another possibility being explored with this instrument is multiplexed MS/MS, where up to 7 ions could be isolated at once and the MS/MS spectrum could be deconvoluted for each selected ion (requires accuracy of <10 ppm). This will be tried with online separations in the near future.

Marvin Vestal (PE BioSystems) described his continuing efforts in MALDI-MS instrument design. The attributes he is aiming for include sensitivity, specificity (resolution, mass accuracy, selective ionization), speed, accuracy, dynamic range, and mass range. The sensitivity will always be limited by chemical noise, but the aim is to reduce the limitations of ionization and data handling. Vestal would like to achieve a sensitivity of 1 fmol with a data acquisition rate of 1 spectrum/s. The instrument he is designing to achieve these aims is a MALDI-TOF/TOF-MS. This system has an ion gate (with 500 resolution and no loss of sensitivity) after the collision cell so that metastable ions created after reacceleration are removed. Although this system is in the early stages of development, the data shown demonstrate that this instrument is meeting most of the stated objectives.

The hybrid quadrupole TOF (Qq-TOF) mass spectrometer developed a few years ago (9), which has now been commercialized, utilizes an ESI for ionization (10). Both Ken Standing (Univ. of Manitoba) and Brian Chait (Rockefeller Univ.) described the use of a MALDI ion source for introduction of ions into a modified commercial Qq-TOF, thus taking advantage of both the high-efficiency ion production of the MALDI and the ion isolation/fragmentation of the quadrupole system with a TOF mass analyzer. Standing presented data showing sensitivity of purified standards (e.g., Substance P) in the 70 amol range (1-min acquisition) for MS and 7 fmol for MS/MS with 10,000 resolution. This instrument offers similar advantages to the TOF/TOF described above.

Online MS analysis of capillary electrophoretic or chromatographic separations of peptide (or proteins) is most often achieved using ESI-MS. Barry Karger (Barnett Institute) described how very small quantities of peptides/proteins could be separated and analyzed using vacuum deposition onto Mylar audio tape for subsequent coupled MALDI-MS analysis. The approach had so far been multiplexed with the effluent of 12 capillaries being deposited under vacuum onto the tape. The approach is designed for high-throughput separations and mass analysis.

Proteomic analyses often employ 2-DE, but David Lubman (Univ. of Michigan) described a liquid-phase 2-D separation of proteins utilizing a novel MS. The requirements of his mass spectrometer were high sensitivity, low duty cycle, and fast response. He designed and built an ion trap to capture ions from the CE coupled to TOF-MS. The 2-D liquid-phase separation consisted of nonporous silica bead RP-HPLC (which provided good resolution <50 kDa) that was conducted at high pH followed by CE and MS. Whole cell lysates were analyzed with this system, and some of the data obtained were presented.

Biological applications. Brian Chait (Rockefeller Univ.) presented the culmination of an enormous amount of work at both the protein chemistry (mass spectrometry) and cell biology levels. The nuclear pore complex (NPC) in yeast is a massive structure (1,000 Å across with 8-fold symmetry) that regulates protein transport in/out of the nucleus. The first step in understanding this structure was to purify the complex and then identify every protein present. The protein fraction was separated by several different chromatographic steps followed by SDS-PAGE from which every visible band was excised and analyzed by MALDI-IT-MS. This was an especially daunting task as the NPC when isolated contains a snapshot of the proteins transiting the NPC at that point in time. Hence, of the 174 proteins identified, 29 were nucleoporins and only 14 were shown to be present in the NPC. These 14 proteins were characterized as being present in the NPC by a variety of techniques. Protein A (4.5 repeats of the Fc binding region) fusions with the proteins of interest were generated, and immunohistochemistry was performed on cells transfected with these constructs. Electron microscopy of hundreds of NPCs following transfection allowed stoichiometry and symmetry (nuclear/cytoplasmic/asymmetric) to be determined. Subcellular fractionation and high-pH extractions were also performed to further characterize localization biochemically. This elegant study has allowed a testable model for nuclear transport to be constructed.

Two examples of the utility of analysis of unfractionated or partially fractionated complex protein mixture digests (see next section) were presented by Scott Patterson (Amgen Inc.). As a first step in the understanding of the interchromatin granule clusters (IGC), a nuclear organelle which is a major site of mRNA splicing. Samples enriched in this structure were digested with trypsin, and the complex mixture of peptides was analyzed by data-dependent LC-MS/MS (8). Some proteins known to be present in these structures were identified together with 19 novel genes (including ESTs). Three of the genes were confirmed to be present in the IGC by immunohistochemistry of cells transfected with yellow fluorescent protein (YFP)-fusion constructs with counter staining of splicing factors. The other study presented identified 108 proteins present in a protein fraction obtained from isolated mitochondria treated with atractyloside [mimicking in vitro the permeability transition pore complex (PTPC) which occurs during apoptosis] (13).

Analysis of immunoprecipitates using a new affinity strategy was presented by Gitte Neubauer (EMBL). The new strategy is referred to as tandem affinity purification (TAP) and was developed by colleagues at EMBL (15). The system utilizes a double tag for higher

specificity and much reduced background. The human spliceosome immunoprecipitated under normal conditions (see Ref. 5 for same approach with yeast tri-snRNP) and using the TAP method were compared, demonstrating the utility of this approach.

The common theme of all of these applications is that MS was utilized early on to provide rapid and accurate protein identifications. The genes identified could then be further analyzed to attempt to determine their function.

The use of MS to identify proteins from 2-DE gels was also described by Al Burlingame (Univ. of California, San Francisco) and Reid Townsend (Oxford GlycoSciences). Burlingame described their work to identify protein targets of acetaminophen during acute toxicity and the intricacies of such analyses (14). Townsend described an Oxford GlycoSciences and Pfizer collaboration to separate by 2-DE and identify proteins from cerebrospinal fluid (CSF) in a study aimed at identifying markers for Alzheimer's disease. CSF is a compartment isolated by the blood-brain barrier but it is not just a filtrate of blood. It is produced by the choroid plexus and has a total central nervous system volume of about 90–150 ml that is turned over a few times per day. Comparative analysis of matched plasma CSF samples (in addition the normal/diseased samples) revealed that key plasma proteins (e.g., albumin, transferrin, IgG) showed markedly different relative ratios between plasma and CSF. For effective 2-DE analysis of these samples, a selective removal of albumin, IgG, transferrin, and haptoglobin had to be developed. This was accomplished by affinity depletion. Interestingly, many features in a 2-DE separation are albumin fragments (in fact, 4% of total features). Their study included 512 samples from 228 patients and resulted in 1,131 features (spots) being annotated. Potential markers of Alzheimer's disease were said to be identified.

Separate from the MS identification issues covered in most of the meeting, Kerstin Strupat (Univ. of Muenster) presented her work on MS analysis of noncovalent complexes. Here the challenge is to transfer noncovalent interactions that occur in the condensed phase to the gas phase. ESI-MS has been shown by a number of groups to work, but MALDI-MS analysis has proved more difficult. Examples of MALDI-MS analysis of noncovalent protein:protein (streptavidin tetramer and the macrophage migration inhibitory factor related proteins MRP-8 and MRP-14) and protein:ligand (aldose reductase:NADP) interactions were presented. Interestingly, analysis of the first laser pulse during a MALDI-MS analysis often allows investigation of noncovalent interactions that are not observed during subsequent pulses (16).

Analysis of complex protein mixtures without gel electrophoresis. The first stage of many proteome projects is the identification of the components comprising the system under study. This is of course the first step in understanding any biological system. As described above, an increasing (but still limited) number of laboratories have access to robotic systems requisite for the analysis of large numbers of spots from 2-DE. However, a trend in the field is emerging toward the elimination of the high-resolution protein separation step prior to protein identification by MS. In this approach, the entire enriched protein fraction is enzymatically digested (usually with trypsin), and the resulting complex peptide mixture is subjected to data-dependent LC-MS/MS. In this approach the peptides are separated by both hydrophobicity (RP-HPLC) and charge (m/z in the mass spectrometer) prior to ion selection by the MS control software (hence, data dependent). At this meeting, presentations from five groups demonstrated the utility of the approach to identify components of complex mixtures.

Analysis of immunoprecipitated proteins or enriched protein fractions (e.g., Golgi complex) by either gel electrophoresis followed by in-gel digestion and MS or digestion of the entire protein fraction and analysis by data-dependent LC-MS/MS using a Qq-TOF was described by Jyoyti Choudhary (Glaxo Wellcome). Batched MS/MS spectra were searched using the Mascot program (**http://www.matrixscience.com**). Data presented showed that if the immunoprecipitate was clean, then direct digestion of the mixture proved slightly more successful than analysis of gel-separated proteins. When an enriched Golgi complex from rat liver was separated by either 2-DE (135 spots) or 1-DE (77 bands) and in-gel digested followed by LC-MS/MS, significantly more proteins were identified from the 1-DE separation.

David Arnott (Genentech) described the proteomics component of Genentech's Secreted Protein Discovery Initiative, which also includes genomic, signal trap, expression, and functional analysis. Arnott evaluated three methods to identify proteins secreted from human umbilical microvascular endothelial cells (HUMECs) into 60 ml of serum-free media; 2-DE and 1-DE (with/without staining) followed by in-gel digestion, and direct digestion of the entire protein mixture. Digests were analyzed using the microcapillary system described above. Interestingly, direct digestion followed by data-dependent LC-MS/MS identified the most proteins, but all three methods were complementary in their hands (21 proteins identified by all three methods but no completely novel gene products).

Analysis of serum fractionated using the Cohn pH/ethanol precipitation protocol followed by digestion of the entire fraction prior to data-dependent LC-MS/MS was described by Karl Clauser (Millennium Pharmaceuticals) in the context of the studies of differences between wild type and ApoE −/− mice. Clauser also presented the bioinformatics flow for data handling, which utilizes a variant of the publicly available MS-Tag (**http://prospector.ucsf.edu/**) for protein identification and a de novo sequence interpretation program referred to as SHERENGA (2). Their stated aim is for searching to keep up with the LC-MS/MS. They have also been experimenting with the IEX ion-exchange protocol developed by Andy Link (7) as a means of decreasing the complexity of the sample and reducing the number of singly charged and highly charged ions as these are less likely to be identified. In one IEX

fraction, 87 plasma proteins were identified in a single run compared with 66 from an unfractionated sample.

Scott Patterson (Amgen) described Amgen's proteomics efforts, now in the third year. They are employing data-dependent LC-MS/MS of complex protein mixture digests. The stated aim is to reduce the complexity such that in an ideal situation only one peptide for each protein in the mixture is fragmented during LC-MS/MS. To achieve this aim, various affinity methods can be employed, and the use of cysteinyl peptide capture using either thiopropyl Sepharose or a biotin alkylating reagent, *N*-[6-(biotinamido)hexyl]-3′-(2′-pyridyldithio)-propionamide (HPDP-biotin), was described (13). The former was used in a large-scale analysis of urinary proteins where digestions of the unfractionated starting material, albumin/IgG depleted, or cysteinyl peptide captured or noncaptured were analyzed. The samples were analyzed with replicate LC-MS/MS runs using narrow mass ranges for ion selection for each run, thereby increasing the number of unique spectra selected for fragmentation. This analysis resulted in the identification of over 200 proteins, including a number of uncharacterized nucleotide sequences (e.g., ESTs). Smaller scale analyses are described above, in one case [soluble intermembrane proteins (SIMPs)] utilizing cysteinyl peptide capture to identify more proteins than with no fractionation. Data handling for this high-throughput effort was also briefly described. A number of the fractions being analyzed have some of the same components; therefore, to enhance the identification process, spectral matching of the database (>5 million spectra) is performed. This links identical spectra and therefore reduces the redundancy associated with re-searching already identified spectra.

Quantitative analysis of two samples without electrophoresis. MALDI-MS, using the surface enhanced laser desorption ionization (SELDI) surface, to search for disease markers in biological fluids was presented by Scot Weinbeger (Ciphergen Biosystems). In this approach, defined chemical/biochemical surfaces are utilized to allow fractionation of proteins from biological fluids in a reproducible manner. This reproducibility allows comparisons between different samples to be made. Weinberger described the search for markers of benign prostatic hyperplasia that, like prostate cancer, displays elevated prostate specific antigen (PSA) levels. The fraction exhibiting a difference between these samples was able to be enzymatically digested, and a number of peptides were generated. These were able to be fragmented using the MALDI Qq-TOF of Standing, described above. It appears as though there is a difference in the relative level of a seminogelin fragment between these two diseases, providing a potential differential marker. The method is sensitive but apparently limited to analysis of proteins less than about 20 kDa (a range not well characterized by 2-DE).

A combination gel/MS approach referred to as a "virtual 2-D gel" was presented by Phil Andrews (Univ. of Michigan). In this approach, proteins are separated by charge using thin-layer isoelectric focusing (IEF), and this gel is then subjected to MALDI-MS. By rastering through the entire IEF gel, a composite display of all acquired MALDI-MS spectra can be generated (hence, the virtual 2-DE). Such analyses would provide very accurate mass measurements, greatly assisting in posttranslational modification analyses as well as potentially quantitation.

Karl Clauser (Millennium Pharmaceuticals) described their efforts at utilizing already existing LC-MS/MS data to attempt to gain some quantitative/qualitative information as to differences between samples. Differences in serum protein levels between wild-type and ApoE −/− mice have been examined using this approach, which compares the MS ion current from peptides identified between LC-MS/MS runs of each sample. Comparison between runs is a difficult task, but data suggested that there is sufficient confidence to state a significant difference if there is a difference of a factor of 3 between some components of the samples.

An LC-MS/MS-based system was described by Steve Gygi (Univ. of Washington) for quantitative analysis of complex mixtures. The technology is referred to as isotope-coded affinity tag (ICAT) (6). The ICAT reagent described here is composed of three units: an affinity reagent (biotin), a linker region (one of two forms), and a reactive group (a thiol-specific reagent, iodoacetic acid). The linker region encodes the mass difference, with the light version having 8 hydrogens and the heavy version having 8 deuteriums. Thus the mass difference is 8 mass units (doubly charged ions will have an *m/z* difference of 4). Following reduction and alkylation of each of the two protein samples with one of the two reagents, the two samples can then be mixed together. All subsequent manipulations are performed as a mixture, culminating in tryptic digestion of the complex sample and capture of the cysteinyl peptides on avidin. The bound peptides are released and analyzed by LC-MS/MS, revealing paired signals of peptides. Calculation of areas under the peak for each paired ion from the LC-MS data provides an accurate record of the relative quantities of the proteins from each starting sample. The MS/MS spectra allow identification of the peptides. The approach was elegantly demonstrated with yeast grown on either galactose-containing media or ethanol-containing media. Proteins expected to be differentially regulated were observed, and, highlighting the advantages of analysis at the protein level as opposed to the mRNA level (e.g., microarray), alcohol dehydrogenase-1 (ADH1) was found to be oppositely regulated (as expected) to ADH2, to which it is 95% homologous. This is a very promising approach for quantitative analysis of complex protein mixtures.

A number of interesting posters were also presented at the meeting, and some of the presenters were given the opportunity to "advertise" their posters. These dealt with the same range of subjects presented in the oral sessions.

Conclusion. The organizers Ruedi Aebersold and John Stults brought together an excellent program for this meeting, with essentially all major laboratories in

this field being represented. The field has grown enormously over the past few years, and advancements presented at this meeting indicate an optimistic view of the future for proteomics. This very successful meeting provided the 162 attendees with the state-of-the-art in mass spectrometry and proteomics.

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a132.099 Examiner copy reference #3

Limits: **Publication Date from 1996 to 1996**

1: Biochem J. 1996 Apr 1;315 (Pt 1):1-9.

Biochem J

The inter-alpha-inhibitor family: from structure to regulation.

Salier JP, Rouet P, Raguenez G, Daveau M.

INSERM Unit-78 and Institut Federatif de Recherches Multidisciplinaires sur les Peptides, Boisguillaume, France.

Inter-alpha-inhibitor (Ialpha I) and related molecules, collectively referred to as the Ialpha I family, are a group of plasma protease inhibitors. They display attractive features such as precursor polypeptides that give rise to mature chains with quite distinct fates and functions, and inter-chain glycosaminoglycan bonds within the various molecules. The discovery of an ever growing number of such molecules has raised pertinent questions about their pathophysiological functions. The knowledge of this family has long been structure-oriented, whereas the structure/function and structure/regulation relationships of the family members and their genes have been largely ignored. These relationships are now being elucidated in events such as gene transcription, precursor processing, changes in plasma protein levels in health and disease and binding capacities that involve hyaluronan as well as other plasma proteins as ligands. This review presents some recent progress made in these fields that paves the way for an understanding of the functions of Ialpha I family members in vivo. Finally, given the wealth of heterogeneous, complicated and sometimes contradictory nomenclatures and acronyms currently in use for this family, a new, uniform, nomenclature is proposed for Ialpha I family genes, precursor polypeptides and assembled proteins.

Publication Types:
- Review
- Review, Tutorial

PMID: 8670091 [PubMed - indexed for MEDLINE]

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Examiner copy reference # 4

1: Clin Chim Acta. 1987 Jan 30;162(2):189-98.

Human inter-alpha-trypsin inhibitor and immunologically related inhibitors investigated by quantitative immunoelectrophoresis. II. Pathological conditions.

Odum L, Hansen-Nord G, Byrjalsen I.

Inter-alpha-trypsin inhibitor (I alpha I) and the immunologically related prealbumin-like-migrating proteinase inhibitor (pA-PI) were investigated by crossed immunoelectrophoresis in sera from 68 persons with myocardial infarction, neoplastic diseases, inflammatory diseases, collagenosis, cirrhosis of the liver or uremia. The concentration of pA-PI in serum increased during each of these diseases (p less than 0.01). The concentration of I alpha I was significantly decreased in patients with cirrhosis (p less than 0.01). In day to day studies of a patient with myocardial infarction, a patient with erysipelas and a postoperative patient the concentration of I alpha I was low normal to decreased in the first days of the conditions and increased thereafter to high normal values. A comparison of the concentration of pA-PI with the excretion of the immunologically identical urinary proteinase inhibitor (UPI) showed that the excretion could not be caused by simple overflow of pA-PI in the kidney. The excretion of UPI followed closely the acute-phase-response, as measured by serum C-reactive protein.

PMID: 3829422 [PubMed - indexed for MEDLINE]

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link[1] ngk)
n.

One of the rings or loops forming a chain.

A unit in a connected series of units: *links of sausage; one link in a molecular chain.*

A unit in a transportation or communications system.

A connecting element; a tie or bond: *grandparents, our link with the past.*

An association; a relationship: *The Alumnae Association is my link to the school's present administration.*

A causal, parallel, or reciprocal relationship; a correlation: *Researchers have detected a link between smoking and heart disease.*

A cuff link.

Abbr. **li** A unit of length used in surveying, equal to 0.01 chain, 7.92 inches, or about 20.12 centimeters.

A rod or lever transmitting motion in a machine.

Computer Science. A segment of text or a graphical item that serves as a cross-reference between parts of a hypertext document or between files or hypertext documents. Also called **hotlink, hyperlink.**

a132.099 Examiner copy reference #6

1: Ann Oncol. 1999;10 Suppl 4:107-10.

Role of tumor markers and mutations in cells and pancreatic juice in the diagnosis of pancreatic cancer.

Tascilar M, Caspers E, Sturm PD, Goggins M, Hruban RH, Offerhaus GJ.

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BACKGROUND: Unresectability at the time of presentation is the most important reason for the poor survival rate of pancreatic carcinoma. Molecular-based tests might improve the early detection of pancreatic cancer at a time when surgical resection is still an option for cure. METHODS: The literature was reviewed concerning the role of molecular-based tests applied to sources other than pancreatic tissue itself, including ERCP-samples, blood and stool, with emphasis on the detection of K-ras mutations and mutant p53 gene product. RESULTS: K-ras mutations have been successfully detected in ERCP brush samples, leading to an increase of the sensitivity and improvement of the diagnostic yield. When pancreatic juice and duodenal fluid are tested for K-ras mutations, the yield is less. K-ras mutations can also be detected in the blood, especially in patients with larger tumors. The presence of K-ras mutations proved also to be useful in discriminating benign and malignant liver nodules, i.e. when during surgery there is suspicion of liver metastases of pancreatic cancer. The accumulation of p53 gene product to immunochemically detectable levels in ERCP brush samples also increases the sensitivity of conventional light microscopy. Other molecular markers such as telomerase and TIMP-1 may prove to be useful too, but await more extensive evaluation. CONCLUSION: Molecular-based tests may be of value in the early detection of pancreatic cancer and might therefore contribute to a better patient survival rate.

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- Review
- Review, Tutorial

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2132.099 Examiner copy reference #7

Limits: **Publication Date from 1992 to 1992**

1: Cancer Res. 1992 May 1;52(9 Suppl):2711s-2718s.

Considerations in bringing a cancer biomarker to clinical application.

Tockman MS, Gupta PK, Pressman NJ, Mulshine JL.

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Specific challenges face our application of emerging biomarkers to early lung cancer detection. These challenges might be considered frontiers to be bridged between established biomedical disciplines, requiring expertise often beyond the range of individual investigators. Cross-disciplinary research already has led to new appreciation of the mechanisms which underlie the phenotypic expression of the transformed cell and places within our grasp the tools which might lead to successful early lung cancer detection. Prior to the successful application of newly described markers, further cross-disciplinary research must (a) refine the selection of biologically appropriate markers, (b) validate such markers against acknowledged disease end points, (c) establish quantitative criteria for marker presence/absence, and (d) confirm marker predictive value in prospective population trials.

Publication Types:
- Review
- Review, Tutorial

PMID: 1563002 [PubMed - indexed for MEDLINE]

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